

ORIGINAL ARTICLE

Association of *Bacillus cereus* Infection with Contaminated Alcohol Prep Pads

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BACKGROUND. *Bacillus* species have caused healthcare-associated outbreaks of invasive disease as well as pseudo-outbreaks. We report an outbreak investigation of blood cultures positive for *Bacillus cereus* associated with alcohol prep pads (APPs) contaminated with *B. cereus* and *Bacillus* species resulting in a rapid internal product recall and subsequent international product recall.

DESIGN. Epidemiologic and microbiologic outbreak investigation.

SETTING. A 300-bed tertiary care children's hospital in Aurora, Colorado.

PATIENTS. Patients with blood or cerebrospinal fluid cultures positive for *B. cereus*.

METHODS. Three patients with blood cultures positive for *B. cereus* were identified in late 2010. Breaches in procedural and surgical techniques, common interventions, and products were explored. The following 3 common products were cultured: sterile saline syringes, chlorhexidine/alcohol skin preparation solution, and APPs. Repetitive sequence-based polymerase chain reaction (Rep-PCR) was used to compare isolates obtained from patients and from APPs and was confirmed by independent pulsed-field gel electrophoresis.

RESULTS. There appeared to be a significant increase in blood cultures positive for *B. cereus* during 2009–2010. *B. cereus* and other *Bacillus* species were cultured from the internal contents of 63.3% of APPs not labeled as sterile, and 8 of the 10 positive lots were manufactured after 2007. None of the isolates obtained from the patients matched strains isolated from the APPs. However, some lots of APPs had strains that were indistinguishable from one another.

CONCLUSIONS. APPs that were not labeled as sterile were contaminated with *Bacillus* species. The product was immediately recalled internally and replaced with APPs from another manufacturer that were labeled as sterile. On January 3, 2011, the manufacturer voluntarily recalled its APPs. Healthcare facilities, healthcare providers, and users of APPs should avoid the use of APPs not specifically labeled as sterile.

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Bacillus cereus is a gram-positive, spore-forming, aerobic bacillus ubiquitous in the environment and well known for causing food poisoning as a result of toxin production.^{1,2} *Bacillus* species, other than *Bacillus anthracis*, have been associated with clinical disease^{3–9} as well as outbreaks of non-gastrointestinal infection, most notably in high-risk patient populations.^{10–14} Pseudo-outbreaks attributable to products and equipment contaminated with *Bacillus* species have also been reported,^{15–26} including contaminated ethyl alcohol²⁷ and cotton²⁸ used to disinfect blood culture bottles. We report an epidemiologic and microbiologic outbreak investigation that identified the contamination of alcohol prep pads (APPs) with

B. cereus and *Bacillus* species and resulted in a rapid internal and subsequent international product recall.

METHODS

Identification of the Problem

In October 2010, a child with newly diagnosed leukemia developed clinical sepsis 24 hours after insertion of an implanted vascular access device (VAD) and required intensive care. Before undergoing the procedure, the child had a peripheral intravenous (IV) catheter and received IV medications. After undergoing the procedure, the child developed extensive, rap-

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idly progressive cellulitis at the VAD insertion site that necessitated surgical debridement, antibiotic therapy, and extended wound management after discharge from the hospital. Postoperative blood, tissue, and wound cultures, in addition to VAD catheter tip cultures obtained during the subsequent VAD removal and debridement surgery, grew *B. cereus*. Because of the unique lot number of the device at our facility and the unusual clinical presentation, this case was reported to the Food and Drug Administration (FDA) via MedWatch. In November 2010, an afebrile infant with congenital heart disease was admitted to the hospital for respiratory distress. A nasal wash performed at admission to the hospital was positive for rhinovirus. Four days after placement of an intrajugular IV catheter for venous access, the infant developed fever and clinical sepsis. Four blood cultures obtained over 2 consecutive days grew *B. cereus*. The hospitalization was extended for treatment with IV antibiotics. Based on the severity of these unusual invasive infections, an investigation was conducted to determine a possible source of the *B. cereus* infections.

Case Definition

A case patient was defined as any inpatient or outpatient with a new blood culture or invasive central nervous system specimen positive for *B. cereus* from October 1, 2010, through November 12, 2010.

Review of Records

Electronic medical, surgical, and microbiology laboratory records were reviewed to identify additional case patients and explore the possibility of common places, persons, times, equipment, procedures, and products for the index case patients identified during October and November. The following 3 common products that were used to treat the index case patients were identified: (1) terminally sterilized 10-mL syringes prefilled with sterile saline solution, for which labeling indicated that the product was sterile and able to be used on a sterile field; (2) sterile applicators packaged with 2% chlorhexidine gluconate/70% isopropyl alcohol solution used for preoperative and procedural skin preparation, for which labeling indicated "Applicator is STERILE if package is intact" (of note, the solution inside the applicator was not classified as sterile); and (3) APPs individually packaged with 70% isopropyl alcohol, for which labeling indicated "Antiseptic; for external use only, for preparation of skin prior to injection," but which were not labeled as sterile or nonsterile.

After identification of the probable source of the outbreak, a review of cases from January 2003 through 2010 was conducted independently by 2 pediatric infectious diseases physicians. Blood and/or cerebrospinal fluid (CSF) cultures or tissue samples positive for *Bacillus* were classified as due to true infection (presence of *Bacillus* species in 1 or more cultures obtained from a patient with a compatible clinical illness

without another explanation), attributable to a probable contaminant (presence of *Bacillus* species in only 1 culture obtained from a patient whose clinical course was inconsistent with bacteremia and/or for whom there was another plausible explanation for the illness), or of indeterminate cause (presence of *Bacillus* species in only 1 culture obtained from a patient whose clinical course could not be categorized as true bacteremia and whose positive result could not be attributed to probable contaminant).

Site Visits and Staff Interviews

Hospital staff involved in the index surgical VAD case were interviewed regarding activities and procedures that occurred before the positive *B. cereus* culture was obtained and to identify deviations in procedural and surgical techniques.

Microbiological Testing and Molecular Typing

The solution of sterile 0.9% saline syringes was injected into blood culture bottles and monitored for 5 days. Three sizes of 2% chlorhexidine/70% isopropyl alcohol applicators were opened, and the internal solutions from the 2 larger-sized applicators and the ampoule and internal solution from the smallest-sized applicators were placed in separate D/E Neutralizing Broth (Becton Dickinson) tubes, incubated overnight, and subcultured to blood agar plates.

The internal contents and outside package of APPs not labeled as sterile from 10 different lots manufactured by Triad Group were cultured on multiple occasions. Both sterile APPs and APPs that lacked sterile labeling from a replacement manufacturer were also cultured. All procedures were conducted in a hood using gloves and sterile instruments. Pads were removed from the package and allowed to air dry to evaporate the alcohol. Pads and packages were individually incubated in tryptic soy broth (TSB) for up to 4 days and subcultured to blood agar plates. Aerobic gram-positive rods were identified as *B. cereus* if they were β -hemolytic, catalase-positive, and motile or as *Bacillus* species if they were not β -hemolytic.

Additional experiments were performed to assess whether pads were contaminated during the process of removal from the package and to determine the bioburden and biodiversity of the isolates by aseptically cutting APPs into multiple sections that were individually cultured. Adherence of organisms to the APPs was measured by immersing pad sections for 15 minutes in 2-mL aliquots of TSB followed by vigorous vortexing and culture of the supernatant.

Repetitive sequence-based polymerase chain reaction (Rep-PCR) technology (Diversilab; bioMérieux Clinical Diagnostics) compared 8 isolates obtained from patients and numerous isolates obtained from APPs using multiple primers (*Bacillus*, *Enterococcus*, *Streptococcus*, and *Staphylococcus*). The Diversilab DNA chip and Agilent 2100 Expert separated amplified DNA into a fingerprint band pattern.

Initial APP culture results and strain typing were inde-

pendently confirmed by the Colorado Department of Public Health and Environment laboratory using similar culture techniques and pulsed-field gel electrophoresis (PFGE) for strain comparison. The PFGE methods were modified from PulseNet protocols (Centers for Disease Control and Prevention [CDC]) to facilitate lysis and macrorestriction digestion of *Bacillus cereus*. BioNumerics (Applied Maths) methods analyzed the gels. Isolates obtained from patients and APPs were considered to be indistinguishable if they were at least 98% similar according to Rep-PCR analysis using bacillus primers and had identical patterns according to PFGE (if tested).

Statistical Analysis

The yearly case frequency from 2003 through 2010 was analyzed using Poisson regression (general linear model; SPSS 19), and proportions were compared with use of the χ^2 or Fischer's exact tests in OpenEpi 2.3.1.

RESULTS

Clinical Epidemiology

Our investigation was triggered by an increased number of blood and tissue cultures positive for *B. cereus* as well as invasive infections in 2 index case patients. APPs were used on the IV catheter hubs of these patients on multiple occasions before the onset of *Bacillus* bacteremia. A third positive culture result, which occurred in late October, was classified as due to a contaminant; in this case, APPs were used only on the diaphragm of the blood culture bottles before injection of the blood specimen into the bottle. Figure 1 shows the yearly distribution of patients with blood and/or CSF cultures positive for *B. cereus* from 2003 through 2010. From 2003 through 2010, there were 36 patients with positive blood culture results and 6 patients with positive CSF culture results. Nineteen (52%) of the blood cultures were obtained in 2009 and 2010. There was a significant ($P = .007$) increase in the number of cases over time (excluding the first quarter of 2011). There was 97% concordance between the independent reviewers, with 1 case adjudicated to indeterminate. The majority (10 of 11) of the 2009 positive cultures were attributable to contaminants, whereas half of the patients in 2010 had true infection. Of the 6 positive CSF culture results, 4 were due to contaminants and 2 (2005 and 2010) were due to true infection.

Recovery of *B. cereus* from the APPs (determined to be a nonsterile Triad-manufactured product) but not from the other products cultured prompted an immediate recall of the APPs at our facility. These APPs had been used exclusively for at least 10 years and were replaced with a different manufacturer's APP, which was labeled as sterile. The Colorado Department of Public Health and Environment notification to the FDA of our investigation prompted a January 5, 2011, recall notice announcing the nationwide recall of all lots of Triad APPs, swabs, and swabsticks because of microbial con-

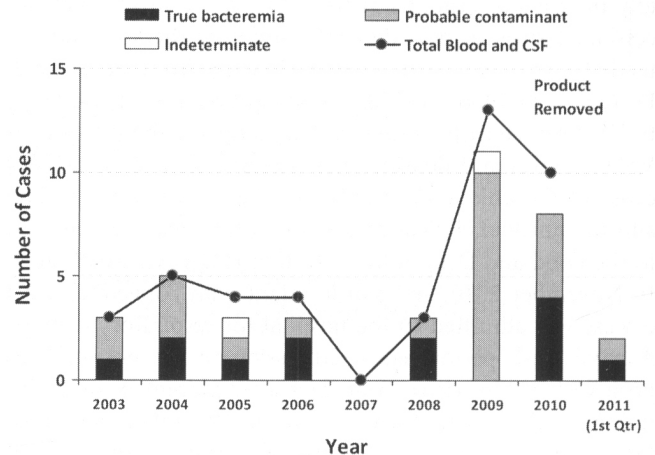


FIGURE 1. Cases in which *Bacillus cereus* was isolated from blood or cerebrospinal fluid (CSF) from 2003 through the first quarter (Qtr) of 2011.

tamination.²⁹ This recall extended internationally, and other Triad product recalls followed. In June 2011, a permanent injunction prohibited the company from manufacturing or distributing drugs or any medical devices until they established a reconditioning plan approved by federal regulators.³⁰

Microbiological Testing and Molecular Typing

Cultures of other products common to the index patients (normal saline syringes and skin prep applicators) yielded no growth. All of the external surfaces of the Triad nonsterile APPs were culture positive for 1 or more *Bacillus* strains, with more non-*cereus* *Bacillus* species isolates than *B. cereus* isolates being recovered. Rarely, staphylococcal or streptococcal species were recovered from the outside of the packages. Overall, *B. cereus* and other *Bacillus* species were consistently cultured from the internal contents of 8 of the 10 lots of the Triad nonsterile APPs, all of which were manufactured during the period 2008–2010, whereas samples from 2 lots manufactured in 2003 and 2005 were tested and had consistently negative culture results (0%; $P < .001$). Culture results for APPs independently cultured by the Colorado Department of Public Health and Environment yielded similar results. *Bacillus* strains were rarely cultured from samples from the initial lot of sterile APPs tested, and such strains were attributed to external contamination during the initial sampling process. Overall, 63.3% of all intact Triad APPs tested grew 1 or more *Bacillus* strains, compared with 3.3% of replacement APPs labeled as sterile ($P < .001$).

There was great diversity among the *Bacillus* isolates obtained from the 8 patients in 2010 and those isolates obtained from the APPs. No matches were found between patients or between patients and APPs. Repeat isolates from the same patient were indistinguishable from each other. Additional primers and/or PFGE did not match several close initial Rep-

PCR typing matches among different patient isolates (Figure 2). Six indistinguishable matches from APP isolates were found among a total of 109 isolates tested. All matches occurred between isolates from different lots, which was confirmed using multiple primers and/or methods. Different strains of *B. cereus* and *Bacillus* species were often isolated

from the same lot and sometimes from different sections of the same pad. Only 2.5% of the 2-mL aliquots of TSB broth from the elution cultures of APPs grew *Bacillus* strains from the eluate, which implies that the organisms were strongly adherent to the pads. Of the segments of various pieces of individual APPs that were individually cultured, 42% grew

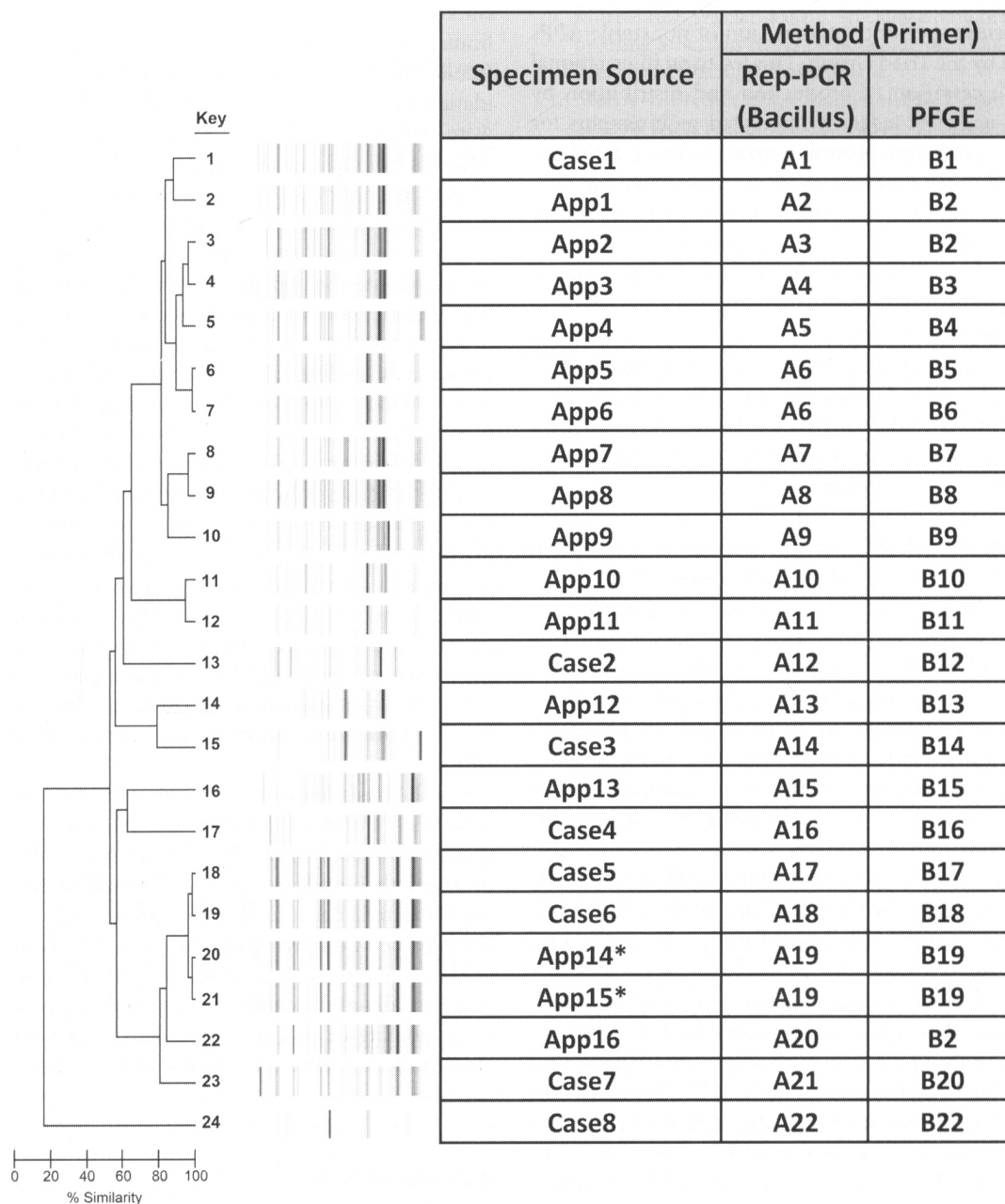


FIGURE 2. Representative repetitive sequence-based polymerase chain reaction (Rep-PCR) dendrogram and fragment pattern demonstrating the number of differences using the Rep-PCR *Bacillus* primer set and pulsed-field gel electrophoresis (PFGE) among 8 case isolates and initial alcohol prep pad (App) isolates. Identical letters and colors in each column designate strains determined to be indistinguishable by that method and/or primer. None of the 8 case isolates were considered to be the same strain, whereas only 2 App isolates from different lots (asterisks) were thought to be indistinguishable using multiple Rep-PCR primers as well as PFGE.

Bacillus strains, with more growing non-*cereus Bacillus* species than grew *Bacillus cereus*, indicating a low but diverse bio-burden within individual pads.

DISCUSSION

We describe an epidemiologic and microbiologic outbreak investigation in response to unusual cases of severe invasive *B. cereus* infection that resulted in the identification of *B. cereus* and *Bacillus* species contamination of nonsterile APPs manufactured by the Triad Group. This led to an international product recall, cessation of production and distribution by the company, and new federally mandated requirements for the facility to meet before resuming manufacturing activities.

B. cereus and other *Bacillus* species are environmental bacteria commonly found in soil, water, and food. These spore-forming gram-positive rods have the ability to survive in harsh conditions, and the spores are resistant to extremes of temperature and to common disinfectants. They can also survive in 70% alcohol solutions.¹ Diseases caused by *Bacillus* species other than *B. anthracis* are usually associated with *B. cereus*, ranging from food poisoning caused by exotoxins produced by organisms contaminating food products to invasive disease in humans, including wound infections, pneumonia, bacteremia and sepsis, and meningitis.

Bacillus species have also been implicated in pseudo-outbreaks of disease related to environmental contamination of blood culture bottles as well as of other hospital products. However, pseudo-outbreaks often include cases of true invasive disease¹⁴ as well, which poses a challenge for clinicians to recognize and correctly categorize *Bacillus* isolates as indicative of true disease versus the probable result of contaminants. A CDC epidemiologic investigation initiated at 24 US health care facilities that had noted an increase in the number of *Bacillus* species isolated from blood cultures during 2007–2008 reported that 75% of the cases were felt to represent device contamination or colonization. The presence of a central venous access device was found to be a risk factor for a blood culture positive for *Bacillus* species (odds ratio [OR], 14; $P < .01$) in the case control study.²⁵ Products were not tested in this study.

We identified 2 index case patients seen over a 3-week period of time with serious cases of confirmed *B. cereus* invasive disease. Within 1 week after identification of the second case, we identified *Bacillus* species in the APPs being used in our hospital. These APPs were not labeled as sterile or non-sterile and were used routinely for disinfection of the skin before injection and to disinfect the top of blood culture bottles before injection of the blood into the bottle. Perhaps most importantly, APPs were used thousands of times a day to “scrub the hub” of needleless catheter hubs before injection of medications, fluids, or flush solutions. We believe that this was the most plausible route of infection in our 2 index case patients.

Extensive studies were conducted to further characterize

those *Bacillus* isolates obtained from patients with invasive disease, compared with isolates obtained from the APPs, although they were not sampled contemporaneously. No APPs were available for culture from supplies that had been used for either of the 2 index patients. Although we did not find any APPs with *Bacillus* isolates that were indistinguishable from isolates found in the blood cultures of any of our patients, we believe that the extreme biodiversity of the organisms isolated from the APPs is a likely explanation for this finding. Prompt notification of the Colorado Department of Public Health and Environment and the FDA and later validation of our microbiologic results by these 2 organizations were critical to this epidemiologic investigation. Removal of all APPs from a large tertiary care pediatric healthcare system proved to be very challenging, because the APPs were widely distributed throughout the organization.

We concluded, but could not conclusively prove, that *Bacillus*-contaminated APPs were the most likely source of true infection in our patients and were also the likely source of the blood culture contaminants that we identified, the latter presumably attributable to alcohol pad disinfection of the top of the blood culture bottle before injection of the patient's blood. This does not preclude other mechanisms of patient exposure and/or culture contamination, because *Bacillus* species are ubiquitous in the environment, and positive cultures have occurred since replacement of the contaminated APPs. We do not know why certain patients developed true invasive disease and others did not. This may relate to differences in virulence between *B. cereus* and other *Bacillus* species or may be attributable to an inoculum influence. Although a high percentage of the Triad APPs cultured grew *Bacillus*, the density of the contamination was relatively low, and our experiments showed that the organisms were quite adherent to the APPs.

On the basis of our experiences with products assumed to be sterile,³¹ but not specifically labeled as such, our healthcare system now uses only APPs labeled as sterile, and we recommend their exclusive use for all procedures for which APPs are indicated. Because *Bacillus* species and other organisms are capable of surviving in what would otherwise be considered disinfecting solutions (eg, 70% alcohol, povidone-iodine, and benzalkonium chloride), it would be prudent to require clear labeling of products intended for patient use as non-sterile unless verified and clearly labeled as being truly sterile.

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